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**14. ABSTRACT**

During the annual contract period, September 22, 2004 to September 21, 2005, the project personnel continued to perform chemical/physical analyses on bulk pharmaceutical substances and formulated drug products, and to develop and (with borrowed labor) manufacture dosage formulations of interest to the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, etc. Specific objectives were to design, develop, validate, and apply methods to determine chemical and physical characteristics of the bulk drugs, drug products, and to determine their stability under defined conditions, and to develop and to manufacture dosage formulations.

**15. SUBJECT TERMS**
Antiparasitic Drugs, Chemical Defense Agents, Chemical Analyses, Stability Studies, Formulation Development, Dosage Formulation Manufacture

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# TABLE OF CONTENTS

# INTRODUCTION

This annual report for Contract DAMD17-03-C-0111 covers the period from September 22, 2004 — September 21, 2005. The report consists of an overview of the major activities, a listing of the specific tasks performed and reports submitted, and description of special projects carried out. The report also includes a listing of personnel receiving pay from this effort and a bibliography of all publications and meeting abstracts that resulted from this contract during the report period.

This contract is concerned with analytical, characterization, and stability studies of chemicals, drugs, and drug formulations, and with development and manufacture of dosage formulations. The studies are monitored by Mr. William Y. Ellis, the Contracting Officer Representative (COR), Chief, Department of Chemical Information, Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR).

The overall objective of this project is the operation of an analytical laboratory to determine the identity, purity, strength, quality, physical and chemical properties, and stability of bulk pharmaceutical substances and formulated drug products, and to develop and manufacture, in limited quantities, dosage formulations of interest of the USAMRMC Drug Development Program for parasitic and infectious diseases, chemical and biological defense, anti-viral studies, etc. Specific objectives are to design, develop, validate, and execute methods to determine the following characteristics of candidate bulk pharmaceutical substances and formulated drugs, and to develop and manufacture, in limited quantities, dosage formulations.

- Identity, purity, and strength
- Stability
- Other physical and chemical characteristics, including weight variation, content uniformity, and other such compendial requirements
- Qualitative and quantitative determination of impurities
- Develop and manufacture, in limited quantities, dosage formulations
- Special projects not covered by the above headings.

## OVERVIEW

During the contract period, September 22, 2004 to September 21, 2005, the emphasis of our project work continues to center about the cGMP production, release, and stability study of the SRI manufactured dosage form of ∝-artesunic acid (AS). An AS unit dosage form consists of two sterile components, the active pharmaceutical substance (APS) and its dissolution medium. The APS component is a vial filled with 110 mg of sterile AS, identified as SRI Batch No. 14462-16, and the dissolution medium is a vial filled 11 mL of sterile pH 8.0, 0.30 M sodium phosphate, identified as Afton Scientific Corp., Batch No. 578-04. In addition, a sterile placebo for AS was manufactured; it consists of a vial filled with 200 mg of sterile mannitol, identified as SRI Batch 14462-28. Of the APS component, 5,550 vials were manufactured and designated by the Army as WR256283;BR29487. Of the dissolution medium component, 8,770 vials were produced and designated by the Army as WR135946;BQ38712. Of the placebo for AS, 2,500 vials were produced and designated by the Army as WR016506;BR29487. After setting aside enough vials of each component for stability studies, the remainder of production lots was released and sent to the Army Repository Laboratory for storage.

As an ongoing part of cGMP manufacturing is a program of stability studies on the manufactured drug product. Stability information is needed for the proper handling and storage of the drug product, and, most importantly, to ensure that the necessary critical characteristics present at the time of production and release can be expected to be present when the drug is administered. A stability protocol, consisting of a stability study schedule, sampling plan, and the tests to be performed for each manufactured drug product component was written, approved, and initiated. For the APS component, the stability protocol was No. 1900.413. For the dissolution-medium component, the stability protocol was No. 1900.415. For the placebo-for-AS component, the stability protocol was No. 1900.414.

Stability protocol No. 1900.413 for the APS component consists of a 6-month accelerated stability study and a 36-month shelf-life stability study. Both studies require the same set of test methods, but not all methods are required for all samplings pulled. Stability protocol No. 1900.415 for the dissolution component consists of accelerated and shelf-life studies of the same durations, and all tests are required for samplings pulled. Stability protocol No. 1900.414 for placebo for AS is similar but truncated to that of the APS component protocol.

For the accelerated stability studies on the APS and placebo components, randomly selected production vials of each were maintained at 40°C / 75% RH over a six (6) months period. After storage of 1, 3, and 6 months, samplings were pulled and tested by the scheduled test methods. Each set of test results were

compared to the time-zero ($T_0$) test results, which are those found at the time the drug product was released. Although the 1- and 3-month results for the APS units under study were invariant from the $T_0$ results, the test methods applied to these two pulled sets of vials did not include what we now considered the most-decisive test for stability, the USP <788> requirement for particulate matter in injections. The test methods scheduled for the 6-month sampling included the test for particulate matter in injections, and APS vials stored 6 months under these conditions failed this test. The placebo units stored identically showed particulate matter counts that easily passed the USP <788> requirements. Accelerated stability study on the dissolution medium component required the first pull after 6 months storage; its results were invariant from those of $T_0$.

Failure of the APS units after 6 months storage at 40°C / 75% RH precipitated a cascade of previously unplanned activities that include re-testing of the 6-month sampling to verify the initial results, investigate the cause(s) of the failure, and to find ways to prevent such failures. These initially unplanned, added activities were the primary focus of the project endeavors for the remainder of report period and beyond.

Resulting from these added, unplanned activities is a great deal of new information. Some are less definitive yet still relevant for which investigations are continuing; others are more defined and are related in numerous reports, but also lead to additional studies.

One of the most thoroughly studied test methods was the determination of particulate matters in injections, USP <788>. The method sample preparation procedure had to be modified to enable the instrument to read only true particulates and not air bubbles and particulates. Our effort was soon put to use and enable the release of the phosphate dissolution medium by its manufacturer, Afton Scientific Corporation, which could not obtain passing results by following USP <788>. In our hands and using our modified procedure, results for the phosphate dissolution medium easily passed the USP <788>.

## SPECIFIC TASKS PERFORMED AND REPORTS SUBMITTED

During the contract period, September 22, 2004 to September 21, 2005, the following tasks were performed and the reports submitted to the COR.

1. WR002976;BS04841, quinine dihydrochloride, an ampoule solution that was a part of a unit dosage of Artesunate For Injection (AFI), WR256283;BQ90150, identification and assay of solution concentration and of dosage, Report 1128.

2. WR016506;BR29478, placebo for artesunic acid (AS), mannitol, SRI Batch 14462-28, one and three months stability, Reports 1103 and 1105.

3. WR135946;BR18064, Afton Scientific Corporation, Batch 578-04, 0.30M, pH 8.0 sodium phosphate, the dissolution medium component of the AS dosage form WR256283;BQ37377, Certificate of Analysis issued.

4. WR227825;BQ39522, determination of solubility and solution stability in water and in plasma, Report 1114.

5. WR249655 (HI-6), WR249943 (MMB-4), and WR253648 (HS-6), determination of solution stabilities as functions of solution pH and of temperatures, Reports 1097 and 1113.

6. WR256283;BR29487, AS, Knoll lot 2.03, a document linking this bulk AS with the SRI manufactured AS drug product, Batch 14462-16, Report 1108.

7. WR256283;BQ36281, AS, lot 2.05, non-milled, WR256283;BQ37377, AS, lot 2.03, milled, WR256283;BQ38641, AS, lot 2.03 milled, determination of 10-month shelf-life stability, Report 1120.

8. WR256283;BQ37377, AS, lot 2.03, determination of room-temperature rate of hydrolysis in 0.30M, pH 8.0 sodium phosphate, Report 1112. Determination of room-temperature rates of hydrolysis in 0.30M, 0.15M, and, 0.10M (all pH 8.0) sodium phosphate, Report 1117.

9. WR256283;BQ37377, ethylene oxide (EtO)-treated bulk AS, determination of two-week, two-, three-, four-, five-, and 10-month shelf-life stability, Reports 1089, 1091, 1093, 1096, 1099, and 1119.

10. WR256283;BQ37377, the AS component in SRI manufactured dosage form, SRI Batch 14462-16, determination of one-, three-, and six-month accelerated stability, Reports 1102, 1104, and 1123A. A failure investigation was performed on units removed after 6-month storage, Report 1118.

11. WR256283;BQ37377, the AS component in SRI manufactured dosage form, SRI Batch 14462-16, determination of one-, three-, and six-month shelf-life stability, Reports 1102, 1104, and 1123A.

12. WR256283, Artesunate For Injection (AFI), manufactured by Guilin No. 2 Pharmaceutical Factory, AS units of this dosage form were analyzed for identity, quality, net content weight (content uniformity), Reports 1100 and 1107. Identically analyzed were the bicarbonate units (dissolution medium) of this dosage form, Report 1109. Impurities in the AS component was characterized, Report 1116. Sterility and endotoxins in both components were determined, Report 1115. Room-temperature rate of hydrolysis of AS in reconstituted media was determined, Report 1112. An overall evaluation of the AFI product appears in Report 1111.

13. WR256283;BQ37377, the AS in the SRI manufactured dosage form, SRI Batch 14462-16 was sterilized by ethylene oxide (EtO) treatment and the residual EtO and its hydrolytic products, chloroethanol (CE) and ethylene glycol (EG), must be determined. Development of a gas chromatographic (GC)/mass spectrometric (MS) assay for EtO and application of the assay to EtO-treated AS, Report 1122. Development of a GC method for EtO in EtO-treated AS, Report 1121. A packed-column GC method for EtO and its application to EtO-treated AS, Report 1124. Application of developed assay to determine EtO, CE, and EG in EtO-treated AS, Report 1120. Determination of rate of loss of EtO in EtO-treated AS dissolved in phosphate, Report 1125. Application of current regulations for residual EtO, CE, and EG in drug products to concentrations of these chemicals found in EtO-treated AS, Report 1126. Validation of GC assay for EtO, CE, and EG in EtO-treated AS, Report 1132.

14. WR288901:BQ39531, solubility and solution stability in water and in plasma, and recovery from plasma, Report 1114.

## SPECIAL PROJECTS

During this report period, the development, refinement, and application of two vital assays required much time and effort. The first of these is the refinement of USP <788>, particulate in injections. Satisfying the requirements in USP <788> is paramount to the “life” of our AS drug product. The sample preparation step given in USP <788> is unsuitable for our drug product because our product dissolution medium, sodium phosphate, is a surfactant and tends for form air bubbles when agitated.

The dissolution of AS in phosphate requires agitation of the mixture because the AS does not easily wet. Agitation of phosphate solutions produces air bubbles, which are counted as particulates in injection in USP <788>. When the bubble count exceeds a certain number, the product fails the USP <788>. On the other hand, soon after the AS dissolves its hydrolytic decomposition begins, resulting in the formation of dihydroartemisinin (DHA). DHA has very limited solubility in aqueous media, including phosphate, and the un-dissolved DHA is counted as particulates. Moreover, an “insoluble” solution is not to be administered. For this reason, the time of AS dissolution needs to minimum, and this is done by optimizing rate of mixture agitation, while keeping the air bubble formation to a minimum. Another factor that needs to be considered in sample preparation is to allow time for the air bubbles that have formed to dissipate before the particulates are measured. This bubble dissipation time has to be long enough for all samples being tested and may need to be adjusted depending on the sample makeup. For example, samples with minimal DHA may require only 10-

15 min dissipation time; samples with “significant” amounts of DHA may require 50-60 min dissipation time.

The need to employ our modified USP <788> was well demonstrated when Afton Scientific Corporation (ASC), manufacturer of the sodium phosphate dissolution medium, employed the published, standard USP <788> to determine particulate in injection in order to release its product. The ASC results failed the USP requirements and the product could not be released. In our hands and using our modified sample preparation procedure, results from the ASC product easily met the USP particulate requirements and was released. Analogously, in the hands of SRI QC personnel, results from the AS constituted in phosphate failed the USP <788>. In our hands and using our modified sample preparation procedure, results from constituted AS solution easily met the USP requirement.

The second special project, which also required a great deal of time and effort, concerns the determinations of EtO and its hydrolysis products in our EtO-treated AS. The sterilization of our bulk AS was performed by a contractor, whose responsibility includes determination of residual EtO and its hydrolysis products. The contractor made an attempt to fulfill this portion of its responsibility, but the attempt fell far short of the goal. Realizing the ultimate burden has to be ours, we developed, validated, applied our methods to determine the subject residues in the EtO-treated AS and applied our results to the existing regulatory requirements.

## PUBLICATIONS AND PRESENTATION

A manuscript entitled “Enhancement and Analytical Method for the Determination of Squalene in Anthrax Vaccine Adsorbed Formulations” was submitted to the COR. Upon receipt of approval, it was submitted for publication in the Journal of Pharmaceutical and Biomedical Analysis.

A poster on “A cGMP Manufactured Artesunic Acid Dosage Formulation” was presented at the APMMC meeting in May 2005.

## AWARDS

No awards were received during the report period.

# PERSONNEL

A listing of personnel who received major contract support during the report period is as follows:

1. Peter Lim, P.I.
2. Ronald Spanggord, Assistant P.I.
3. Patrick Macauley, Chemist
4. Mindy Johnson, Chemist
5. Schridhar Hegde, Chemist II
6. Steve Kim, Chemist I
7. Helen Parish, QC supervisor
8. Ami Penticoff, Document Specialist
9. Irina Beylin, Senior QC Manager
10. William Opsahl, QA Director

## SUMMARY/CONCLUSIONS

During the early part of the annual contract period, September 22, 2004 to September 21, 2005, the main emphasis was to complete the manufacture of the artesunic acid dosage form, its placebo, and its vehicle for dissolution. A total of 5,500 vials of the active, 2,500 vials of placebo, and 8,770 vials of the dissolution vehicle were manufactured, released, and delivered to the Army. As a part of the cGMP manufacturing, accelerated and shelf-life stability studies on the three components were initiated and continuing. Detailed comparisons of chemical/physical properties of the Guilin Artesunate for Injection and the artesunic acid dosage form we manufactured were made. Problems related to stability of the SRI manufactured dosage form constituted the major effort of the contract year.

Efforts unrelated to artesunic acid include studies on oximes.

Respectfully Submitted:

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